Chapter 6
Summary

Rotavirus (RV) remains a main concern for neonatal diarrhea, overwhelming mainly in the developing countries of Asia and Africa. As per the recent reports, South Asian country like India endured significant child deaths of 78,583 children per year. Besides, RV outbreaks have also resulted immense economic losses in the livestock industry and here, group A rotaviruses (RVA) acquire the crest for frequent epidemics in piglets and calves. The viruses, having a segmented genome can exchange genetic material, more likely during mixed/co-infections by human-human or human-animal strains. Since, rotaviral transmission is mainly through fecal-oral route and is known for their environmental resilience, it is tempting to suggest that close association of animals with their human handlers favors an appropriate situation for interspecies/zoonotic transmission. Although without clear evidence of host restriction, animal RV gene segments were detected from humans or vice-versa globally. Therefore, such region holds a potential hub for surfaced new infections and provides an evidence for animals to act as a source of virus and/or of genetic material transmission leading to emergence of novel/unusual genotype combinations. The subsequent global spread of these apparent unusual/novel strains may result in the establishment to the population through time representing a challenge to the massive vaccine evaluation programs. The national surveillance network programs 2005 accomplished by ICMR in collaboration with CDC initiated again in 2012 which is ensuring representative data from various new clinical recruitment sites or hospitals. Nevertheless, many of the high disease burden areas in India portraying rural subsistence with lack of sanitary disposal maintenance system and human-animals close settings remains unexplored. Therefore, we aimed to investigate the rotavirus diversity prevailing in several characterized regions of Barak and Brahmaputra valleys. The study area represented agrarian provinces where animal is reared for customary activity for
living and are housed indoors in group-housing or straw-lined sheds or pens, which allows easy contact with waste matter for the human handlers and vice versa. Thus, the animals along with the dwellers, both are at risk of contracting infection which is maintained in the environment. Such unique animal–human mixing patterns add for potential inter-species transmission and genetic reassortment within the rotaviruses generating diversity on many such occasions. Therefore, surveillance of circulating rotavirus strains is an essential endeavor to support such program.

We characterized rotaviruses from 500 diarrheal stool specimens of children, piglets and calves during 2011–2014 collected from different outbreak areas and hospitals of different geographical regions of Barak and Brahmaputra valleys of Assam. The samples were initially screened through monoclonal antibody based enzyme immunoassay (mAb-EIA) followed by RNA-PAGE, VP7-VP4 gene amplification by reverse transcription PCR (RT-PCR) and G-serotyping by nested-multiplex PCR. The overall prevalence of rotavirus was found to be 28.2% where positive cases from piglets were of 37.9% followed by children and calves having 24.8% and 29.6% respectively. Majority of samples demonstrated characteristic group A rotavirus (RVA) electropherotype of 4:2:3:2 pattern by RNA-PAGE. Moreover, RNA profiles of 11 samples from piglets and calves revealed variation in the migration pattern of their class II, III and class IV segments. The study, for the first time from the valley, detected neonatal RVA positive cases from human and animal sharing similar setting. Moreover, almost all the strains were found to be non-typeable by nested-multiplex PCR G-serotyping.

To examine further the rotavirus genetic diversity based on VP7 and VP4 genes, restriction fragment length polymorphism (RFLP) assay was developed. The assay involved direct digestion of RT-PCR amplified VP7 and VP4 cDNAs with five restriction enzymes (VspI, HaeIII, NlaIV, HindIII and TaqI) independently and were compared with the global isolates. 48 RFLP patterns were identified for all the strains, and of these, 20 patterns were associated with local
isolates. A positive assortment of intrinsic serotypes from multiple host origin and certain single or combined enzyme profiles was highly dominant in the population. Significant genetic variations were established between global and Indian isolates and none, of the RFLP patterns were shared among them. These data suggested Indian wild type rotavirus population was distinguishable based on these genes and co-circulation of distinct strains among different hosts is foremost, indicating the possible likelihood of interspecies transmission.

The non-typeable strains demonstrated during G-serotyping were genotyped based on their VP7 and VP4 sequences. Analyzing the sequences, the common G-P combinations detected was G1P[8] (25%), followed by G9P[8] (5%). Notably, unusual G-P combination G1P[7] were observed in significant numbers (35%) from porcine and bovine. Rare combinations viz., G1P[8] from porcine; G1P[13], G1P[23], G3P[7] from porcine and bovine and G5P[8] from human were also observed, though at lower levels. Successive unexpected detection of porcine-like rotavirus strain isolated from a human sample and 12 rotaviruses of human lineages from animal species provided a clear evidence of possible human-animal reassortment during the period. Thus, based on the two structural genes VP7 and VP4, the study provides direct evidence to support the interspecies transmission and reassortment of human and porcine/bovine rotaviruses.

Two live-attenuated rotavirus group A (RVA) vaccines, Rotarix (G1P[8]) and RotaTeq (G1-G4, P[8]), have been successfully introduced in many countries worldwide, including India, though through private sector. At present, little is known about the relationship between currently circulating rotavirus strains and the vaccine strains. Comparisons of the local isolates to the existing vaccines resolved the antigenic relationship among themselves. Among all the non-vaccine VP7 and VP4 components of local RVA strains, increased number of mutations in the active binding sites were detected which can successively modify the antigenicity of the respective region. The precise impact of amino acid changes in the antigenic epitope predicted during the study provided strong evidence
regarding the differences of the circulating RVA strains to the existing rotavirus vaccines at amino acid level. Over time, these differences might result in selection for strains that escape the RVA neutralizing-antibody pressure induced by vaccines.

The present work thus, is a limelight regarding identification of common rotavirus strains complying vaccine components along with noteworthy cases of unusual/rare genotypes from man and animals neonates during 2011-2014. The study is also useful to describe substantial cases of inter-species/zoonotic transmissions as ascertained by prevalence of intrinsic G or P-type of unusual combinations in multiple hosts having relationship with prototype strains of countries like China, Srilanka, Thailand etc. Thus, enormous genetic diversity and evident cases of unusual strains with their subsequent antigenic disparity to existing vaccines may assist in the evolution of rotaviruses and consecutive emergence of novel RV strains, presenting a challenge to the efficacy of G1-G4 based vaccines. Moreover, the present work provides an epidemiological data for year 2011-2014 to the current National Surveillance Network System, India.

The study thus reinforces the need to continue national surveillance program including farm animals because of (i) their close contact with humans, especially in developing countries; (ii) increased reports of the detection of strains common to animals in human populations; and (iii) the increasing spread and detection of strains with unusual G and P types and untypeable strains. Moreover, the study warrants the national surveillance programs to include this geographical region of the country where rotavirus diversity hotspots have been discovered throughout the study. Thus, surveillance programs and characterization of rotavirus strains shall continue specifically for these unusual rotavirus strain combinations lacking common rotavirus VP4 and VP7 proteins, which have been the focus of future rotavirus vaccine development.